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Research Article

HYPOGLYCEAMIC ACTIVITY OF FRACTIONS OF ETHYL ACETATE FRACTION OF THE WATER EXTRACT OF *ARTOCARPUS HETEROPHYLLUS* SENESCENT LEAVES

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ABSTRACT

The aqueous extract of the senescent leaves of *Artocarpus heterophyllus* is traditionally used in Sri Lanka as a hypoglyceamic agent. It has been previously reported that the hypoglyceamic activity lies in the ethyl acetate fraction of the water extract (EA/W). The EA/W fraction was fractionated by chromatography on sephadex LH-20. Five fractions eluting with the following solvent systems were obtained. Fraction 1, dichloromethane/ hexane (4:1), Fraction 2, dichloromethane/acetone (3:2), Fraction 3, dichloromethane/ acetone (1:4), Fraction 4, dichloromethane/ methanol (1:1) and Fraction 5, methanol. These fractions were screened for *in vivo* hypoglyceamic activity. Each fraction was tested for its effect on the blood glucose levels of fasted normal and diabetic rats. None of the fractions caused hypoglyceamia on normal rats. However there was significant ($p < 0.001$) reduction in the blood glucose level during the first three hours after giving the fractions 3, 4 and 5 to diabetic rats. Among the three fractions fraction 4 showed the highest hypoglyceamic activity. Fractions 3, 4 and 5 were also the most active in the glucose tolerance test carried out on both normal and diabetic rats. However fraction 3 showed the highest activity in this assay. In both assays using diabetic rats fractions 3, 4 and 5 at 50 mg/kg body weight showed activity of the same order as glibenclamide at 5 mg/ kg body weight. These results maybe of potential use for the development of an anti diabetic drug from leaves of *Artocarpus heterophyllus*.

KEYWORDS: *Artocarpus heterophyllus,* Diabetes mellitus, Hypoglyceamic activity, Sephadex LH-20.

INTRODUCTION

Diabetes mellitus is a syndrome of chronic hyperglyceamia due to relative insulin deficiency, resistance, or both[1]. During the last twenty years the prevalence of the disease has increased all over the world and it now affects all populations. The International Diabetes Federation (IDF) estimated that in 2013 worldwide 387 million people were suffering from diabetes mellitus with projections that the number will increase to 592 million by 2035 [2]. The prevalence of diabetes and its adverse health effects has risen more rapidly in South Asia than in any other large region of the world [3]. In 2011, 8.3% of the adult population or 71.4 million South Asians were estimated to have diabetes. In 2013 Sri Lanka diabetes prevalence has reported 8.0% which means that 1.1 million people are suffering from diabetes mellitus [3]. Long-term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers leading to amputations and charcot joints, and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction[4]. Further, diabetic patients have an increased rate of atherosclerotic, cardiovascular, peripheral arterial and cerebrovascular disease, hypertension and abnormalities of lipoprotein metabolism[4]. Mortality and morbidity are increased due to these complications. Currently insulin and various oral antidiabetic agents are used as monotherapy or in combination to achieve better glyceamic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects and most of drugs show development of resistance[5]. It is still a challenge to manage diabetes mellitus without any side effects. The search for new drugs which are safer and more effective is an active area of research. *Artocarpus heterophyllus* is a well-known plant which belongs to family Moraceae and its leaves are used ethnomedically in Sri Lanka for the control of diabetes mellitus. The antidiabetic and hypoglyceamic effects of the leaf extract have been demonstrated in animal models and in human subjects[6-10]. A study on a rat model shows that the hypoglyceamic activity of the ethyl acetate fraction of the water extract of the leaves at a dose of 50 mg / kg body weight was greater than that of tolbutamide, a sulphonyl urea drug commonly used for treatment of hyperglyceamia at a dose of 15 mg / kg body weight [7,11]. In this paper we report our work on evaluating the potential of *A. heterophyllus* leaves for the treatment of diabetes mellitus through its *in vivo* hypoglyceamic activity of the fractions of ethyl acetate fraction of the water extract.

MATERIALS AND METHODS

Plant materials

Senescent leaves of *A. heterophyllus* were collected from a plant (cultivar *Waraka*) growing in a home garden in Wijerama in the Colombo district. The Material was authenticated by Mr. Isuru Kariyawasam of the Department of Botany, University of Sri Jayewardenepura and a voucher specimen (B7/006(SJP)) has been deposited in the herbarium, Department of Botany, University of Sri Jayewardenepura. These leaves were washed and dried on air for 3 hours and crushed using a mechanical blender and used for extractions.

Extraction

Ethyl acetate soluble fraction of the water extract of *A. heterophyllus* leaves (EA/W)

A sample of senescent leaves of *A. heterophyllus* (500 g) was extracted by refluxing with 2500 mL of distilled water in a 5 L round bottom flask for 4 hours. The extract was filtered through a cotton wool plug fixed to a funnel while hot and allowed to cool to room temperature. The extract was concentrated under vacuum until the total volume was 5 mL. Excess ethanol was added to precipitate the high molecular weight fraction. The mixture was filtered and the filtrate was concentrated under vacuum until the total volume was 500 mL and extracted with ethyl acetate (300 mL x 6) in a separatory funnel. The ethyl acetate extracts were combined and the solvent was evaporated under reduced pressure to obtain a brownish-black sticky solid (1.2 g) (EA/W).

Fractionation of EA/W

Sephadex LH-20 was soaked in hexane: dichloromethane (1:4) overnight and packed it into a column (30 g, 1.6 cm x 30 cm). Sticky solid EA/W (1.2 mg) was soaked to 1 g of Sephadex LH-20 and placed on the top of the column. Column was eluted with 5 different solvents and fractions were collected separately. Fraction 1 was eluted with dichloromethane/hexane 4:1 (1 L) and fraction 2, 3, 4 and 5 were eluted with dichloromethane/ acetone 3:2 (1 L), dichloromethane/acetone 1:4 (0.9 L), dichloromethane/ methanol 1:1 (1 L) and methanol (1 L) respectively. The weights of each fraction after evaporation of the solvent were obtained and distribution of compounds were analyzed by TLC (Silica (GF 254) with solvent systems; ethyl acetate: dichloromethane: methanol: formic acid (58:38:2:2) and ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27).

Animal model

Male Wistar rats approximately 8 - 12 weeks old, weighing 150 - 300 g from Animal Centre, HEJ Research Institute, Karachi, Pakistan were used for the study. The animals were housed in standard cages, three per cage with autoclaved sawdust as bedding material. They were fed pelleted standard rat feed and water *ad libitum*. Rats were maintained at 12 hours light/dark cycle at room temperature. They were individually identified by colour markings on their body. The rats were handled in accordance with the standard guide for the care and use of laboratory animals and necessary skills for rat handling were obtained before commencing the research.

For *in vivo* studies on normoglyceamic rats 42 rats were divided into 7 groups (groups A -G) and for *in vivo* studies in the diabetic model experiment 48 rats were divided into 8 groups (groups H - O). The animals used were kept fasted overnight before commencement of experiment.

Induction of diabetes mellitus

Rats in groups H – O were fasted overnight and nicotinamide was given intraperitonially (120 mg/ kg body weight). Then after 15 minutes streptozotocin was given intravenous (60 mg/kg body weight). Normal food and 5% glucose (instead of water) was given for 24 hours *ad libitum*. After that until two weeks normal food and water was given *ad libitum*. The rats were fasted overnight and their fasting blood glucose levels were measured to check whether diabetes has been induced (> 120 mg/ dL).

Effect on blood glucose levels in normoglyceamic rats

Animals in groups A – G were fasted overnight and fasting blood glucose levels of the rats were measured. After that group A served as control and received water. Group B received a single dose of 50 mg/ kg body weight EA/W. Groups C, D, E, F and G received a single dose of fraction 1,2,3,4 and 5 (50 mg/ kg body weight) respectively which were obtained from sephadex LH-20 column. All samples were given orally. After that blood glucose levels were measured at 1 hour, 2 hours and 3 hours after administration of sample. Blood was obtained from the tail vein. Blood glucose was measured using a glucometer (Accu-Check glucometer)

Effect on fasting blood glucose levels in diabetic rats

Diabetic rats of groups H – O were fasted overnight and fasting blood glucose levels of the rats were measured. Group H served as control and received water. Group I received a single dose of 50 mg/ kg body weight EA/W. Groups J, K, L, M and N received fraction 1,2,3,4 and 5 (50 mg/ kg body weight) respectively which were obtained from Sephadex LH-20 column respectively. Group O received glibenclamide at 5 mg/ kg body weight. All samples were given orally. After that blood glucose levels were measured at 1 hour, 2 hours and 3 hours after administration of sample. Blood was obtained from the tail vein. Blood glucose was immediately measured using a glucometer (Accu-Check glucometer).

Effect on glucose tolerance in normoglyceamic rats

After 2 weeks of the experiment using normoglyceamic rats, animals in groups A – G were kept fasted overnight and fasting blood glucose levels of the rats were measured. After that group A served as control and received water. Group B received a single dose of 50 mg/kg body weight EA/W. Groups C, D, E, F and G received a single dose of fraction (50 mg/kg body weight)1,2,3,4 and 5 respectively which were obtained from Sephadex LH-20 column. All samples were given orally. After 1hour blood glucose level was measured and a glucose load (3 g/kg body weight) was administrated. After that blood glucose levels were measured at 1 hour, 2 hours, and 3 hours after the administration of glucose load. Blood was obtained from the tail vein. Blood glucose was immediately measured using a glucometer (Accu-Check glucometer).

Effect on glucose tolerance in diabetic rats

After 2 weeks of the experiment diabetic rats, animals in groups H – O were kept fasted overnight and fasting blood glucose levels of the rats were measured. Group H served as control and received water. Group I received a single dose of 50 mg/ kg body weight EA. Groups J, K, L, M and N received a single dose (50 mg/kg body weight) of fraction 1,2,3,4 and 5 respectively which were obtained from Sephadex LH-20 column. All samples were given orally. After 1hour blood glucose level was measured and a glucose load (3 g/kg body weight) was administrated. Blood glucose level was measured at 1 hour, 2 hours and 3 hours after the administration of glucose load. Blood was obtained from the tail vein. Blood glucose was immediately measured using a glucometer (Accu-Check glucometer).

RESULTS AND DISCUSSION

The fractions (1 -5) obtained from the Sephadex LH- 20 column were tested for their hypoglyceamic activity to identify active fractions. The effect of the fractions on the glucose levels in fasted rats, with and without the administration of a glucose load was tested. Both normal and streptozotocin induced diabetic rats were subjected to these tests.

Treatment of the rats with nicotinamide and streptozotocin resulted in elevated plasma glucose levels consistent with diabetes (>120 mg/ dL), whereas non-treated rats without nicotinamide and streptozotocin had normal glucose levels (< 120 mg/ dL).

Effects on blood glucose levels in normoglyceamic rats after administration of fractions of EA/W

According to the results obtained neither EA/W nor the fractions brought about any significant lowering or increasing of the blood glucose levels. This indicates that these samples do not lead to hypoglyceamic activity in the normal rat. This is an advantage in antidiabetic drug as it reduces the risk of hypoglyceamia.

Effect on blood glucose levels in diabetic rats after administration of fractions of EA/W

Figure 1 describes the variation of blood glucose levels with time after administration of fractions of EA/W and the Figure 2 shows the reduction in blood glucose level with time for the different fractions.

It can be seen that there was no significant change in the blood glucose level of the control group during the three hours. Surprisingly in fraction 1 there was an increase in the blood glucose level in the 1st hour which then came back to the starting level after the second hour and lowered at the 3rd hour. Fraction 2 did not show any significant change ($p > 0.1$) in the first and second hour after administration of fractions of EA/W. But at the 3rd hour it showed significant reduction ($p < 0.01$) with respect to control group. Figure 2 clearly show that the highest reduction occurs at second hour after administration of the samples. Also it shows fraction 3, 4 and 5 have significant reduction ($p < .001$) with respect to control group. Fraction 4 showed the highest reduction at the second hour after administration of sample. When comparing with EA/W fraction 3, 4 and 5 have significant enhancement ($p < 0.01$) in hypoglyceamic activity over parent EA/W at first and second hours. Fraction 4 shows significant reduction even at the third hour ($p < 0.01$) over the parent EA/W. When comparing with glebenclamide, fractions 3, 4 and 5 show a significant reduction in blood glucose level ($p < 0.01$) at second hour of administration of fractions. It should be noted that the dosage of glibenclamide was one tenth of the dose of the fractions.

Effects on blood glucose levels in normoglyceamic rats with the administration of a glucose load (Glucose tolerance Test)

Figure 3 shows the variation of blood glucose levels with time and Figure 4 shows the change in blood glucose level with respect to the control (Percentage reduction in blood glucose level with respect to normal control). The more sensitive time for evaluating the effects of the fractions will be when the blood glucose level is at its highest. In all groups the highest increase in blood glucose level is observed at 1st hour after glucose load, after which it reduces over a period of 2 hours and almost back to the starting level.

The percentage reduction of the increase in blood glucose level caused by the different fractions at first hour after glucose load was calculated as below.

$$\text{Percentage reduction of the increase in blood glucose level with respect to control} = \frac{(BGL_{c1} - BGL_{c0}) - (BGL_{t} - BGL_{t0}) \times 100\%}{(BGL_{c1} - BGL_{c0})}$$

BGL_{c0} – Blood glucose level of control 1st hour after administration of water immediately before the glucose load.

BGL_{c1} – Blood glucose level of control 1 hour after administration of glucose load.

BGL_{t0} - Blood glucose level of test group 1st hour after administration of sample immediately before the glucose load.

BGL_{t1} – Blood glucose level of test group 1 hour after administration of glucose

It should be noted that our method of calculating the effect of the test substances in lowering blood glucose is more accurate than the commonly used method of calculating it using the equation

$$\text{Percentage reduction in blood glucose level} = \frac{(BGL_{c} - BGL_{t}) \times 100\%}{BGL_{c}}$$

As it compensates for the difference in the initial blood glucose levels between the control group and the test substances groups.

Calculated percentage reduction of the increase in blood glucose level of the fractions of EA/W at 2 hours after administration of sample was given in Figure 4 and it show that all the fractions except fraction 2 and fraction 1, show leased glucose elevation after glucose load. The EA/W was the fraction which had lowest glucose reduction curve with the time. Fraction 2, 3, 4 and 5 also have reduced blood glucose level time curve than the normal control rats.

Effects on blood glucose levels in diabetic rats with the administration of a glucose load (Glucose tolerance Test)

Of the five fractions obtained from sephadex LH-20 column fractions 1 and 2 did not show activities. As a result only the fractions 3,4,5 and EA/W were subjected to the study.

The results obtained are given in Figure 5. The results clearly show that all fractions (Fraction 3, 4, 5 and EA/W) have ability to reduce the post load of glucose than the control. As in normal rats, the highest blood glucose level was observed at the 1st hour after the glucose load. However the rate of reduction in blood glucose level thereafter was much slower than in normal rats. Therefore the EA/W shows the maximum percentage reduction with respect to control. Comparing the three fractions maximum percentage reduction of blood glucose is seen in fraction 3. Thus, there is no enhancement of activity in the fractions.

Although the composition of the three fractions were different from each other and from EA/W as analyzed by thin layer chromatography, there are no significant difference ($p>0.01$) in the activities. This suggests that EA/W contains many different compounds having approximately the same activity.

When comparing all the *in vivo* results we can clearly see that optimum time of action is at the end of the 2nd hour after administration of sample.

CONCLUSION

The ethyl acetate fraction of the water extract of the senescent leaves of *A. heterophyllus* was fractionated into five fractions using Sephadex LH-20. Fractions 3, 4 and 5 were the most active for *in vivo* studies. Fraction 4 showed highest activity in reducing blood glucose levels, while fraction 3 was the most active in the glucose tolerance test, indicating that fraction 3 contain a glucose uptake inhibitor. These results maybe of potential use for the development of an anti diabetic drug from leaves of *Artocarpus heterophyllus*.

FIGURES

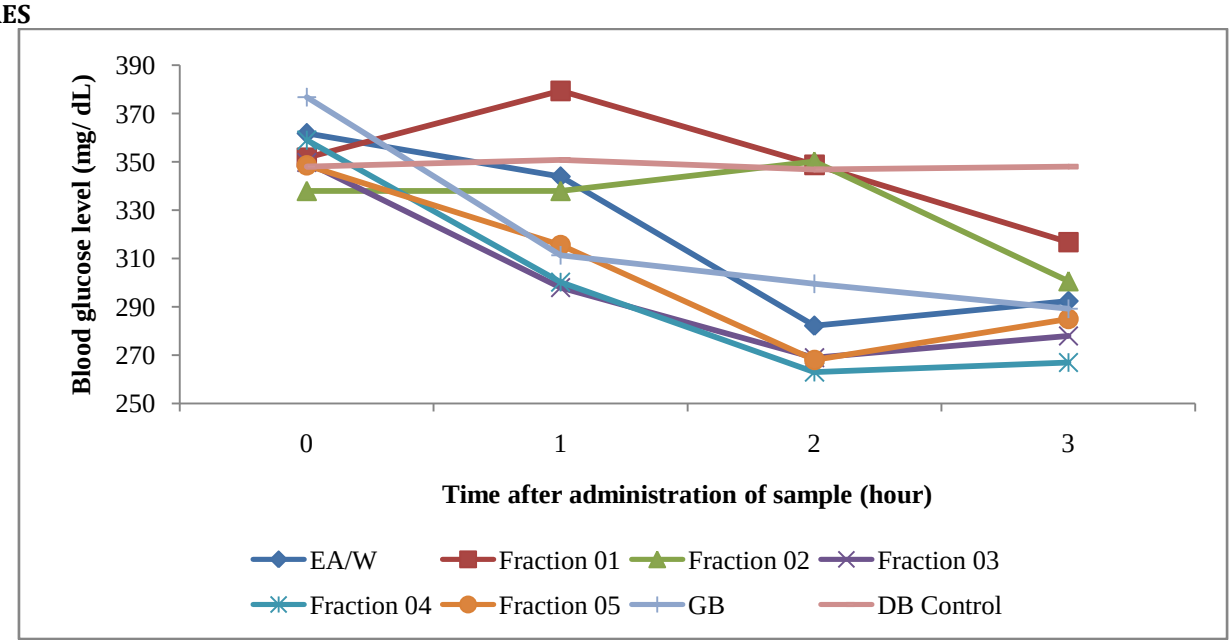

Figure 1: Variation of blood glucose levels with time in diabetic rats after administration of fractions of EA/W. (Value at t=0 correspond to fasting blood glucose level)

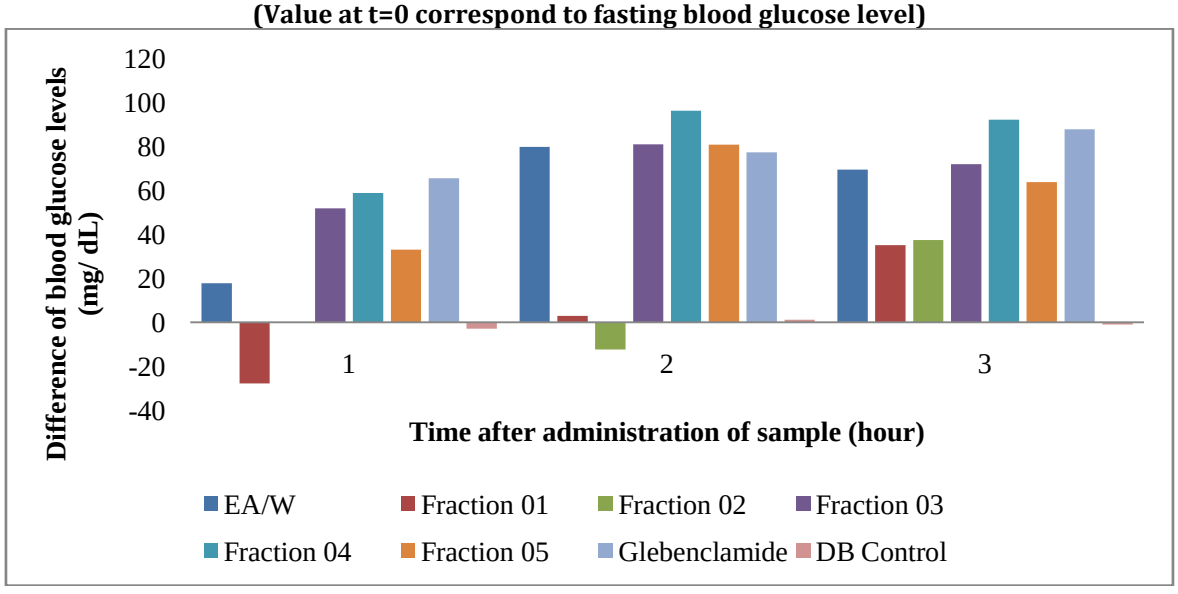

Figure 2: Reduction in blood glucose levels with time in diabetic rats after administration of fractions of EA/W.

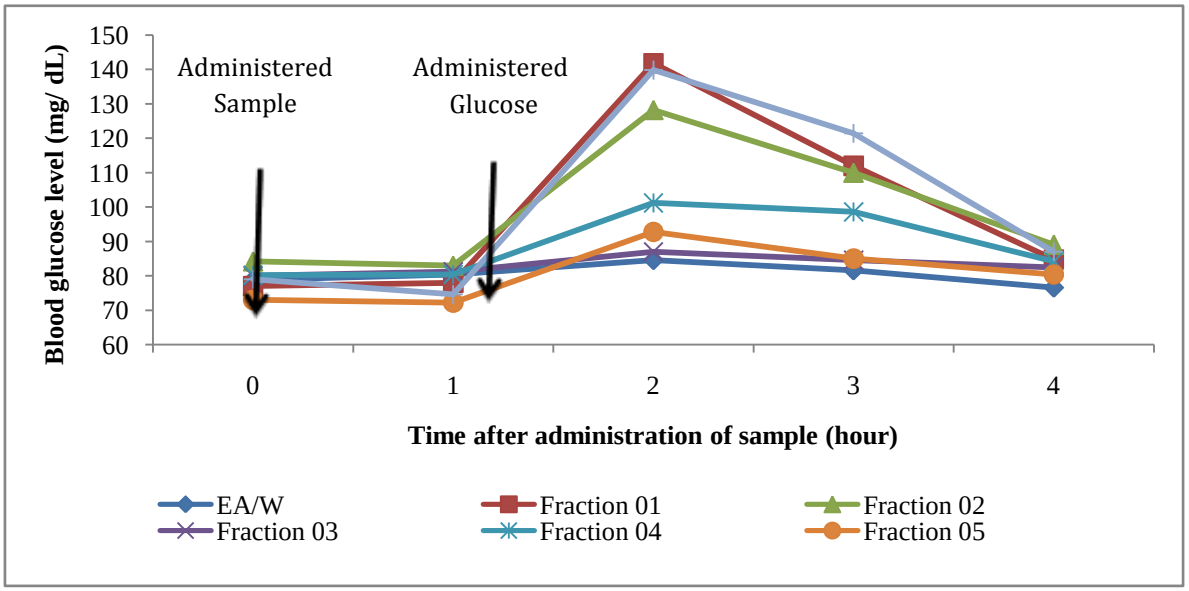

Figure 3: Variation of blood glucose levels of normoglyceamic rats with the time after administration of sample followed by glucose load.

Value at t=0 correspond to fasting blood sugar level

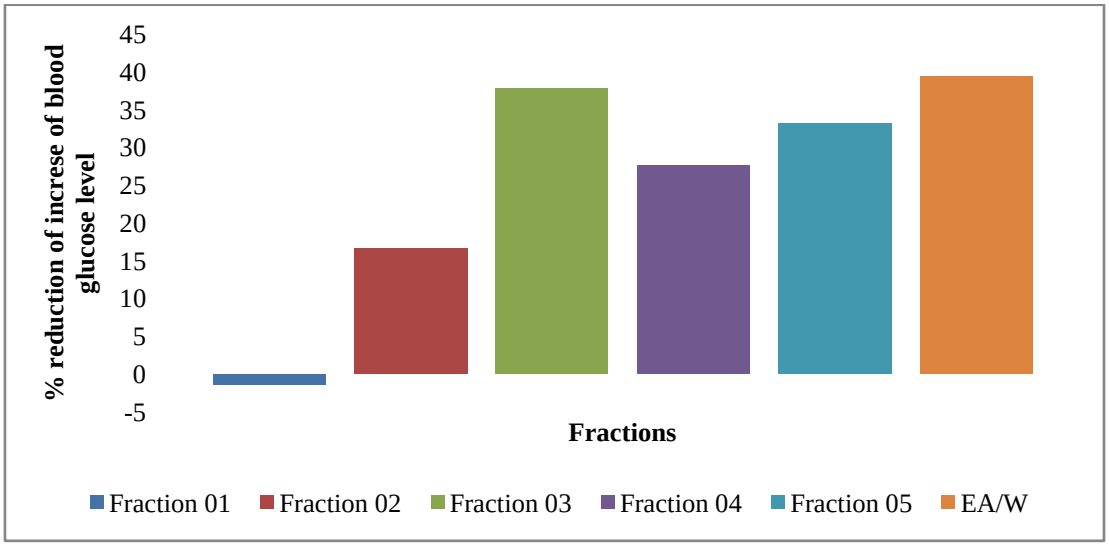

Figure 4: Percentage reduction of blood glucose level of normoglyceamic rats with glucose load with respect to control one hour after glucose load

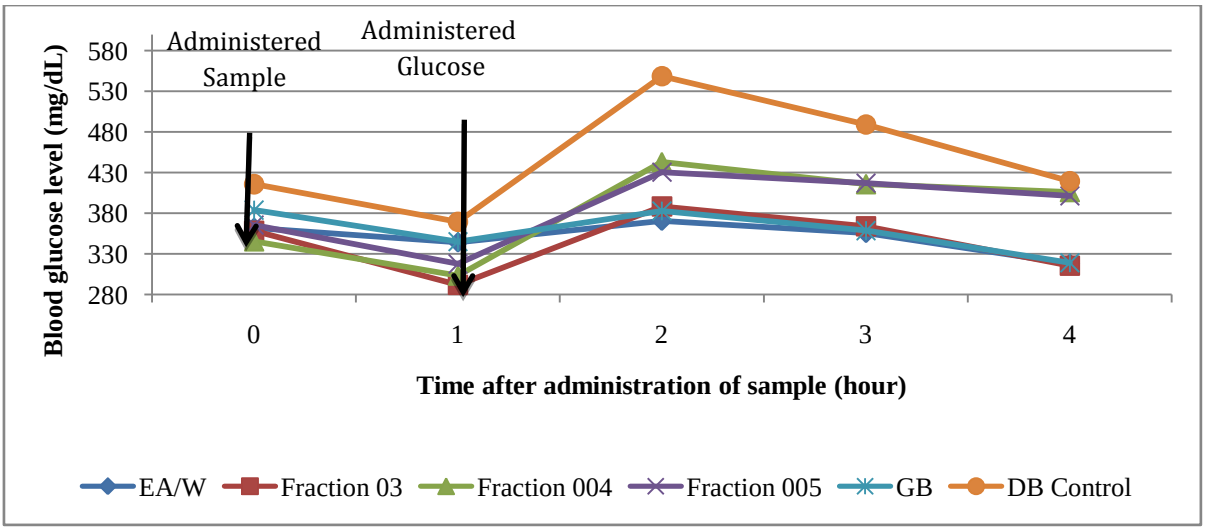

Figure 5: Variation of blood glucose levels of diabetic rats with time after administration of sample followed by glucose load

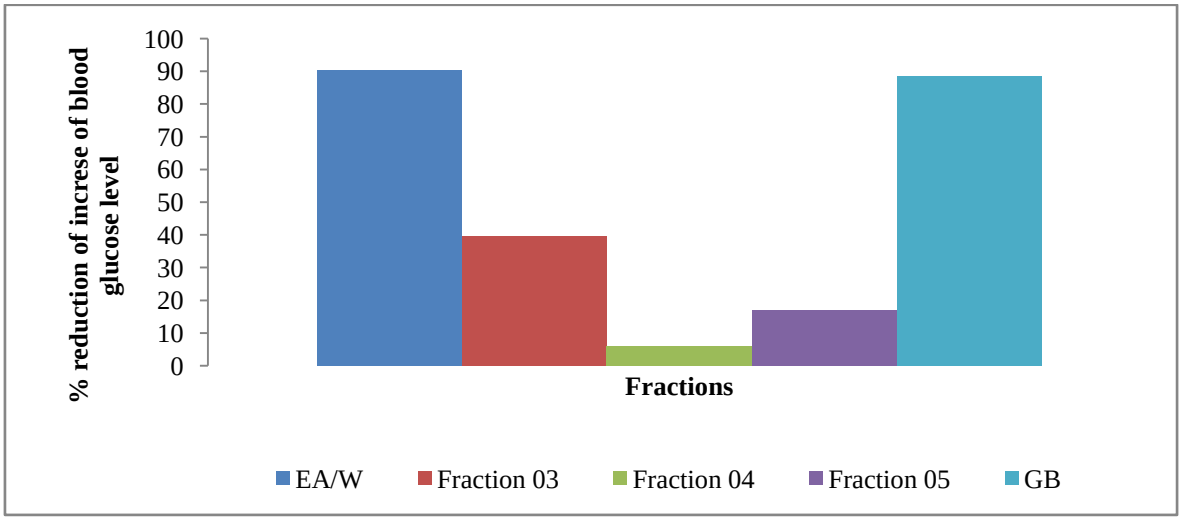

Figure 3.8: Percentage reduction of increase in blood glucose level of diabetic rats with glucose load with respect to control one hour after glucose load

REFERENCES

1. Kumar, P. & Clark, M,. 2009, Kumar & Clarks's Clinical Medicine, Saunders, Elsevier, Spain.
2. International Diabetes Federation, 2013, Diabetes Atlas, 6th edition. Brussels: Available at www.idf.org/diabetesatlas
3. Ghaffar, A., Reddy, K. S. & Singhi, M., 2004, Clinical review: Burden of non-communicable diseases in South Asia, BMJ Journals, vol. 328, April, pp. 807 - 10.
4. American Diabetes Association, 2014, Standards of Medical Care in Diabetes 2014, Diabetes Care, vol. 37, no. 1, January, pp. S14 - S78.
5. Jung, M., Park M., Lee, H. C., Kang, Y. H., Kang E. S. and Kim, S. K., 2006, Antidiabetic Agents from Medicinal Plants, Current Medicinal Chemistry, vol. 13, pp. 1203 - 1218.
6. Chandrika, J. G., Fernando, W. S., Wickramasinghe, S. M. D. N. & Wedage, W. S., 2005, Effects of pro proanthocyanidin and flavonoid fractions of Jack leaves (Artocarpus heterophyllus) on the blood glucose levels in healthy, male Wistar rats, Gampaha Wickramarachchi Ayurveda Institute, Yakkala, vol. 2, pp. 26 - 29.
7. Chandrika, U. G., Wedage, W. S., Wickramasinghe, S. M. D. N. & Fernando, W. S., 2006, Hypoglyceamic action of the flavonoid fraction of Artocarpus heterophyllus leaf, African Journal of Traditional Complementary and Alternative Medicines, vol. 3, no. 2, pp. 42 - 50.
8. Fernando, M. R. & Thabrew, M. I., 2001, Extra-pancreatic effects contributing to the hypoglycaemic activity of Artocarpus heterophyllus, The Ceylon journal of Medical Science, vol. 44, pp. 1 - 10.
9. Fernando, M. R., Thabrew, M. I. & Karunanayake, E. H., 1990, Hypoglyceamic activity of some medicinal plants in Sri-Lanka, General Pharmacology, vol. 21, pp. 779 - 782.
10. Fernando, M. R., Wickramasinghe, S. M. D. N., Thabrew, M. I., Ariyananda, P. L. & Karunanayake, E. H., 1991, Effect of Artocarpus heterophyllus and Asteracanthus longifolia on glucose tolerance in normal human and maturity onset diabetic patients, Journal of Ethnopharmacology, vol. 81, pp. 277 - 282.
11. Chackrewarthy, S., Thabrew, M. I., Weerasuriya, M. & Jayasekera, S., 2010, Evaluation of the hypoglycemic and hypolipidemic effects of an ethyl acetate fraction of Artocarpus heterophyllus (jak) leaves in streptozotocin-induced diabetic rats, pharmacognosy magazine, vol. 6, no. 23, pp. 186 - 190.

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